REPORT of the SHORT TERM SCIENTIFIC MISSION, COST FA1208

Title: Histological Response of Potato to the cyst nematode *Globodera pallida* pathotype Pa1

Start date: 28th February 2016 **End date:** 13th March 2016

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Host: Miroslaw Sobczak, Warsaw University of Life Sciences

Background:

Attracted by root exudates, the potato cyst nematode *Globodera pallida* juvenile invades the root of its host. Once inside it moves intracellularly toward the vascular cylinder whereupon it induces the formation of its feeding site. This specialised feeding site, known as a syncytium, is a multinucleate cell produced by multiple rounds of mitosis without cell division. It becomes the sedentary nematode's only source of nutrients.

In response to the constant threat of pathogen infection, plants have evolved resistance pathways to combat these threats. There are several mechanisms of nematode resistance which have been observed *in vivo* but all are classified based on the appearance of a hypersensitive response (HR). For example, with the root-knot nematode *Meloidogyne* spp., the HR response with *Mi* in tomato occurs at the site of nematode entry into the root, and involves a programmed cell death (PCD) around the infection site as well as the initiation of plant defence responses to restrict the nematode's movement. More commonly in *Globodera* and *Heterodera* species a delayed-HR is observed where the nematode has the ability to induce a syncytial feeding site, but is followed by the slow deterioration or sometimes abnormal development of it.

Due to these nematodes being endoparasitic (spending most of their lifecycle within the root), the timing of a HR is unclear. For *Meloidogyne* spp. the necrosis can be seen within two days, with cellular changes observable in as little as eight hours (Giebel (1982). In contrast, the resistance response mounted against the citrus nematode *Tylenchulus semipenetrans* can take almost two weeks to develop.

Little is known about the mechanism underlying the *H2* resistance pathway which controls resistance toward the Pa1 pathotype of *G. pallida.* The aim of this STSM was to determine whether the *H2* response was targeted to the syncytial feeding site, at the cells surrounding the feeding site, or the cells associated with the nematode migration through the root.

Objectives/ Purpose of the visit:

Through the use of both light and transmission electron microscopic techniques, visualise, image, and analyse the development and degradation of the nematode feeding site in susceptible and resistant potato cultivars.

Description of the work:

Before travelling to Warsaw, stem samples of susceptible cultivar Desirée and resistant P55/7 potato genotype were micropropagated and cultured on MS30. After three weeks of growth, the roots of each plant were inoculated with 50 sterilised Pa1 second-stage juveniles. Root samples were collected at 1, 5, 7, 10 and 14 dpi (days post infection), sectioned around the potential infection site and fixed in Karnovsky fixative for two hours. While travelling, samples were stored in 50mM sodium cacodylate buffer.

Upon arrival, samples were washed four times in 50mM sodium cacodylate buffer, each wash lasting 10 minutes. Samples were then stained with 2% osmium tetroxide in 50mM sodium cacodylate for two hours. This was followed by four washes in 50mM sodium cacodylate buffer, each wash lasting 10 minutes. After washing, samples were dehydrated using a 10% graded ethanol series, each step lasting 10 minutes. Samples underwent a substitution step with propylene oxide, each substitution lasted 20 minutes. To infiltrate samples with EPON resin they underwent a gradient, substituting the propylene oxide for resin at 10, 25, 50, 70, 90, and two 100% solutions. Each step lasted two hours, with the 70% and second 100% step being left overnight. Samples in resin were transferred to embedding moulds and underwent polymerisation at 65°C for 16 hours.

Embedded samples were trimmed and sectioned to 3µm (semi-thin), and stained with 0.5% Toluidine blue for analysis under light microscopy, and 90µm (ultra-thin) and stained with both uranyl acetate and lead citrate for analysis under transmission electron microscope (TEM).

Main results:

Comparison of resistant and susceptible root after inoculation

<u>24 hours post-inoculation</u>

A total of three infection sites were collected and sectioned for light microscopy for the resistant genotype P55/7. Nematodes were not visible on the root surface, but damage to epithelial cells signaled potential nematode infection. Observation under the light microscope did not show any syncytial formation (Figure 1), leading to the conclusion that the nematode was still migrating through the root toward the vascular cylinder.

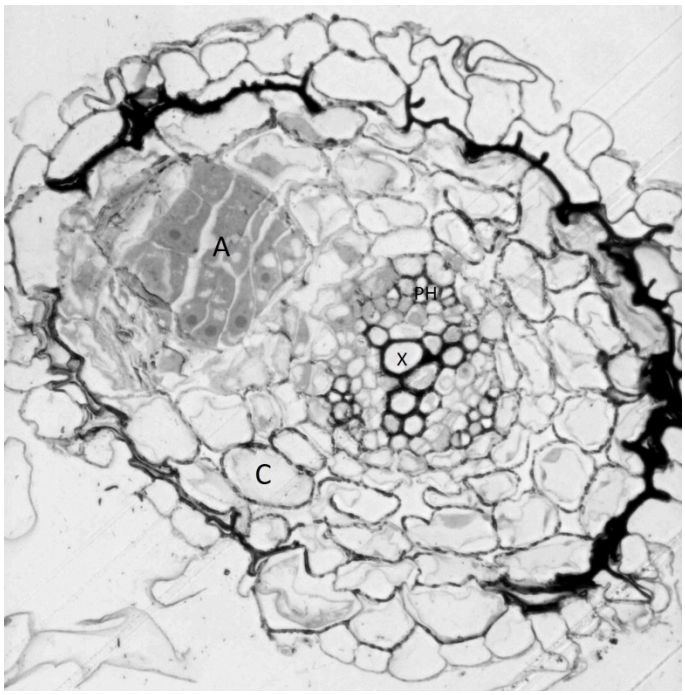

Figure 1. Light microscopy image of resistant P55/7 root, 24 hours post infection. *A* apical root, *PH* phloem bundle, *X* Xylem. *C* Cortex

5 days post-inoculation

Three samples from both Desirée and P55/7 were collected and sectioned for light microscopy, with sections being taken from two of the Desirée samples for observation under electron microscope. One of the Desirée samples showed plasmolysis in the cells, and no sites similar to a potential syncytium so was discarded (Figure 2A), The P55/7 sections showed enlargement of cambial cells, which is linked to the start of syncytium formation (Figure 2B). However, upon further analysis under the electron microscope (Figure 3), no cortex bridge was visible between the epidermal cells and the vascular cylinder, and the enlarged cambial cells did not show a decrease in cytoplasmic density, leading to the conclusion that they were not the start of syncytial formation.

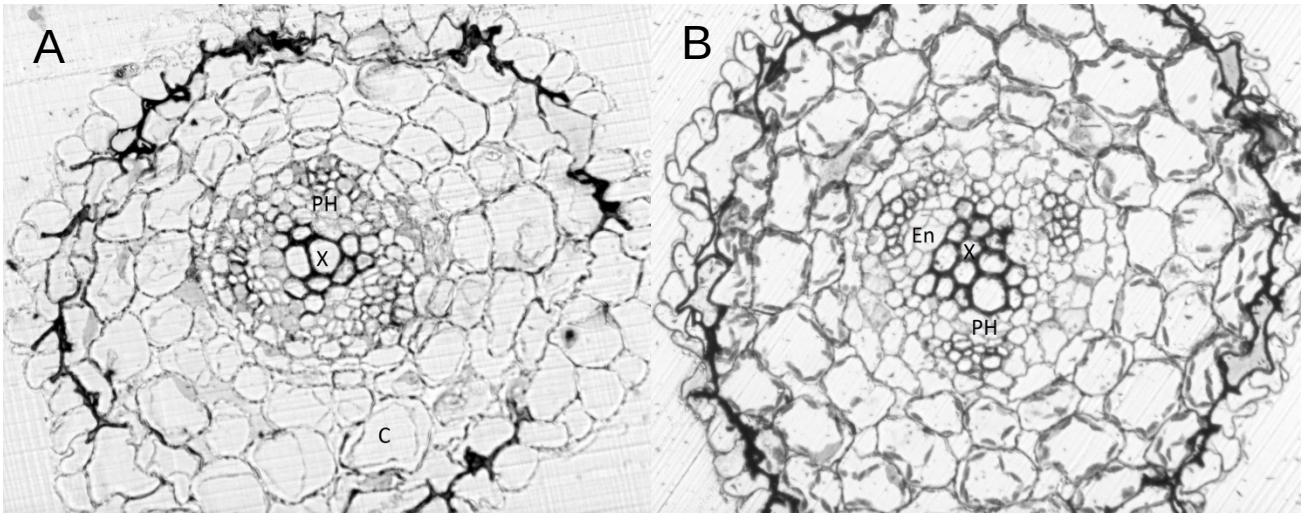

Figure 2. Light microscopy images of A: Susceptible Desirée root, 5 days post infection. B: Resistant P55/7 root, 5 days post infection. *PH* Phloem, *X* Xylem, *C* Cortex, *En* Enlarged Cambial Cell

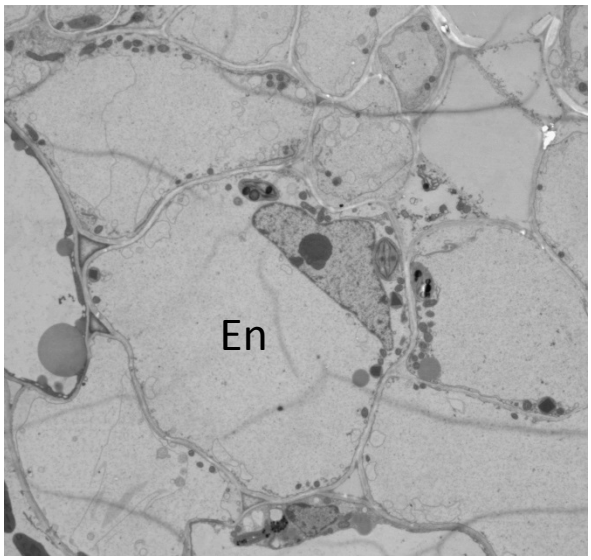

Figure 3. Transmission Electron Microscopy image of enlarged cambial cell (En).

7 days post-inoculation

Two samples were successfully sectioned for the resistant P55/7, and a total of six for Desirée. The resistant sample (Figure 4A) showed no symptoms of infection; the xylem is undergoing secondary formation, and the cambial cells and phloem bundles are uninhibited in their growth and expansion. In Figure 4B the lower cambial cells are expanded and more round, instead of flat stacked cells which are normally present. This could potentially be the start of a cortical bridge formation; further semi-thin sectioning will be carried out and any changes in the cambial cells will indicate potential syncytial formation.

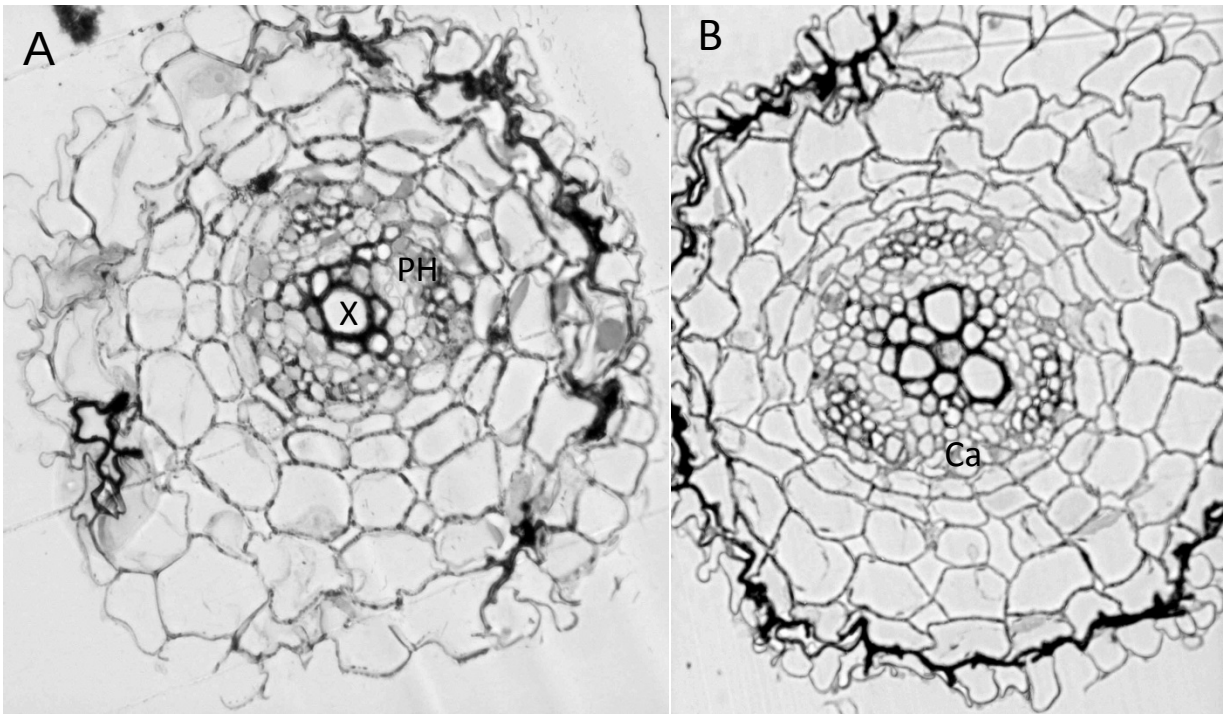

Figure 4. Light microscopy images of A: Resistant P55/7 roots, 7 days post infection. B: Susceptible Desirée roots, 7 days post infection. *X* Xylem, *PH* Phloem, *Ca* Cambial Bundle Cells

10 days post-inoculation

The P55/7 samples which were harvested and sectioned (Figure 5A) showed no sign of infection. This could mean that the sample wasn't sectioned deeply enough to visualise the syncytium, or that abnormalities on the root surface which resulted in it being harvested, were not abnormalities linked to nematode infection, but perhaps other forms of mechanical damage within the root. The same is true for Figure 5B which also shows no signs of infection.

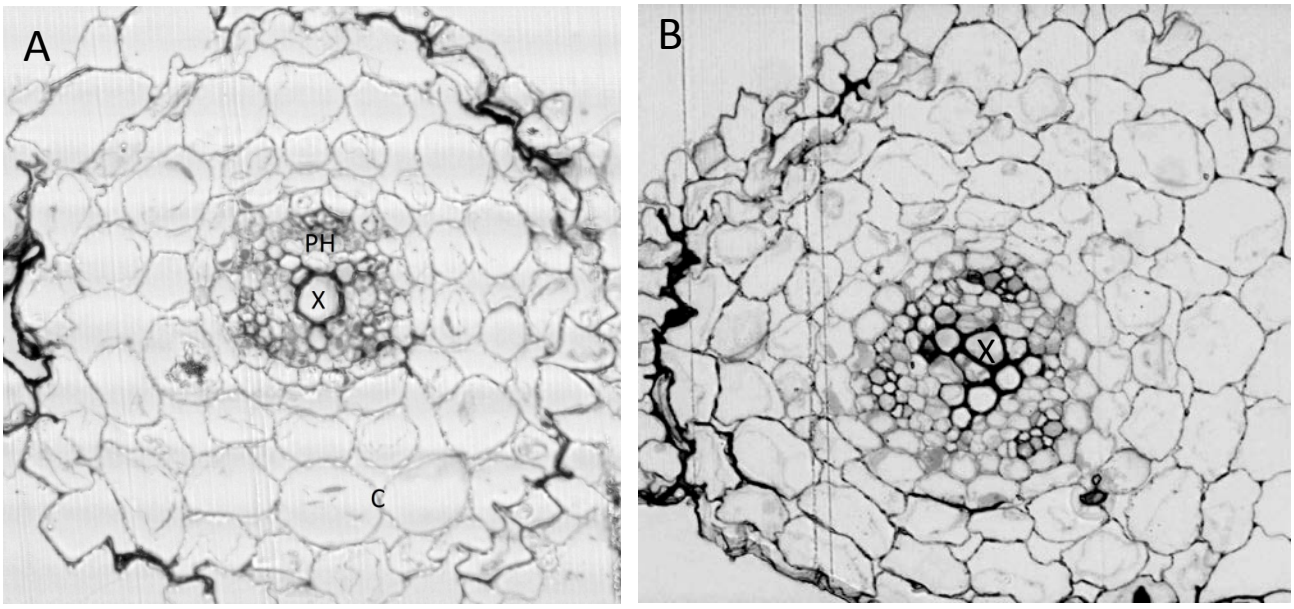

Figure 5. Light microscopy images of A: Resistant P55/7 root, 10 days post infection. B: Susceptible Desiree root, 10 days post infection. *X* Xylem, *PH* Phloem

14 days post-inoculation

Contamination on the Desirée plate meant samples could only be harvested from the P55/7 plate. Again samples showed no sign of infection (Figure 6), but proper development of the root.

The samples which were harvested and taken to Warsaw unfortunately did not show any signs of syncytial formation. However, the training in histological techniques with Prof Sobczak was invaluable. Further infections of resistant roots and histology of potential infection sites will be performed at JHI in order to fully fulfil the objectives of this scientific mission.

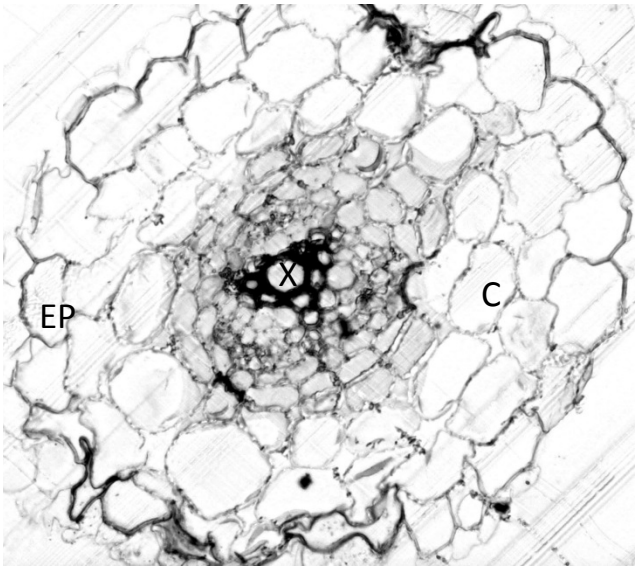

Figure 6. Light microscopy image of Resistant P55/7 root cells, 14 days post infection. *X* Xylem, *C* Cortex, *EP* Epidermis

Projected publications/articles related to or resulting from the STSM:

Once the syncytium has been successfully imaged in both resistant and susceptible root systems an article will be published which outlines; the timeline for Pa1 nematode infection to occur, and the timeline and appearance of a *H2*-induced resistance response.